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SPRUCE BUDWORM POPULATION-DAMAGE STUDY

Progress Report
1960

By

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TABLE OF CONTENTS

	Page
General Objectives	1
Study plots	1
Larval development and defoliation	2
Objectives	2
Methods	2
Results	3
Biological control	12
Objectives	12
Methods	12
Results	13
Damage	13
Objectives	13
Methods	13
Results	14
Discussion	21
Appendix	23

INDEX OF TABLES

		Page
Table 1.	Tree species composition by age class of spruce budworm study plots	2
Table 2.	Budworm population by instars at time of sampling, (1958-1960)	4
Table 3.	Parasites and percent parasitism within the spruce budworm larval and pupal populations (1958-1960)	7
Table 4.	Table for field use of prediction of total seasonal defoliation based on immature larval populations per 15-inch twig	9
Table 5.	Field operative form for prediction of immature (2-3-4 instar) budworm larvae based on emerged pupal cases per 15-inch Douglas-fir twig	11
Table 6.	Comparison of 1959 and 1960 egg mass data (Montana)	12
Table 7.	Sums for the defoliation by tree age interaction	14
Table 8.	Sums for the tree age by individual tree interaction	14
Table 9.	Total needle complement by defoliation class	15
Table 10.	Needle ranks and method for determining the needle complement value (NCV) of Douglas-fir	16
Table 11.	Needle complements and needle complement values of green, non-defoliated trees and different degrees of defoliation for six consecutive years	17
Table 12.	Needle complements and needle complement values of study trees by site and percent defoliation occurring in 1960	18
Table 13.	Analyses of variance for differences in the regressions of needle complement between green and defoliated trees	20

INDEX OF FIGURES

		Page
Figure 1.	Larval and parasite populations and defoliation (1958-1960)	5
Figure 2.	Regression of total seasonal defoliation on immature budworm larval populations	9
Figure 3.	Regression of immature (2-3-4 instar) budworm larvae on emerged budworm pupal cases in Douglas-fir	11
Figure 4.	Rate of needle retention between nondefoliated and defoliated Douglas-fir trees	19
Figure 5.	Regressions of rate of needle retention on age of needle for Douglas-fir	20

GENERAL OBJECTIVES

The year 1960 was the fourth year of general biological research on spruce budworm, Choristoneura fumiferana (Clem.) populations in southern Idaho. Intensified and more restricted studies should be undertaken on phases of the budworm problem.

The general objectives of the studies were (1) to gather information on the development of spruce budworm populations to improve biological evaluation techniques, and (2) to relate spruce budworm populations to damage and answer the questions, "what constitutes a damaging budworm population," and "at what point does actual damage to tree occur?" The over-all study was approached and reported by phases of the insect development and damage. Thus, specific sub-objectives are stated for each phase.

Techniques and methods have been developed from work on the first objective. Work toward the second objective has produced ideas and theories more than answers. Future work would relate to the second objective only.

This report presents a summary of results over the past four years, recounts the objectives, accomplishments, and indicates problems for consideration in future programs.

STUDY PLOTS

Three 1/10-acre plots were established within three types of infestation for intensive population studies.

Plot EN-1. Growth consisted primarily of uneven-aged Douglas-fir that had been infested since 1949. The budworm population displayed rather strong fluctuations from year to year.

Plot EN-2. Growth consisted primarily of uneven-aged Douglas-fir that became lightly infested in 1957.

Plot EX-4. Growth consisted primarily of uneven-aged alpine fir that had been infested since 1955.

All trees within each plot were marked and classified as to species, d.b.h., tree height, crown length, position in the stand, age class, and vigor.

The stand composition for each plot is shown in table 1.

Table 1.--Tree species composition by age class in spruce budworm study plots

Plot	Tree species	TREE AGE: Mature %	Mature No.	Immature %	Immature No.	Pole %	Pole No.	Sapling %	Sapling No.	Reprod. %	Reprod. No.	By plot %	By plot No.
EN-1	DF	9	3	13	4	31	10	38	12	9	3	91	32
	Lpp	33	1	0	0	33	1	33	1	0	0	9	3
EN-2	DF	14	3	45	10	9	2	18	4	14	3	85	22
	Lpp	25	1	0	0	25	1	50	2	0	0	15	4
EX-4	AF	8	2	28	7	28	7	16	4	20	5	83	25
	DF	0	0	25	1	0	0	50	2	25	1	13	4
	Lpp	100	1	0	0	0	0	0	0	0	0	4	1

LARVAL DEVELOPMENT AND DEFOLIATION

Objectives

1. To sample larval, pupal, and parasite populations, and seasonal defoliation to determine trend of infestation.

2. To identify and inventory parasites and evaluate their effect on the larval and pupal populations.

3. To develop methods for either single or multi-visit sampling in order to evaluate the sampled population, relate it to defoliation within different tree age classes, and determine if data could be used to forecast populations the next year.

4. To determine the best combination of temperatures and length of exposure to break diapause of overwintering larvae.

Methods

A sample unit consisted of four 15-inch twigs per tree, one terminal and three laterals. This unit was used for sampling all larval and pupal populations, and determining defoliation ratings. Samples were not removed from saplings and reproduction. Because of limited twig complement, pruning these smaller trees for samples would have affected the populations involved and made it difficult to obtain records each year.

The early larval stages were sampled when approximately 50 percent of the population had emerged from hibernation and mined buds or needles (2nd - 4th instars). The majority of individuals collected were dissected for parasite inventory. Late larval and pupal stages were to be sampled when approximately 50 percent pupation had occurred. Individuals collected were reared to maturity, or if they succumbed to parasitism the emerging parasites were inventoried.

Seasonal defoliation was based on the number of damaged buds (new growth tips) on the 15-inch twig. Defoliation classes were as follows:

(1) 90 percent or more of the needles destroyed per bud.
(2) Between 75-90 percent of the needles destroyed per bud.
(3) " 50-75 " " " " " " "
(4) " 25-50 " " " " " " "
(5) Less than 25 " " " " " " "
(6) Residual undamaged buds.

Results

The results in the sampling and analyses are based on the 90 percent confidence level. Budworm larval populations follow the negative binomial distribution, and in order to perform the usual analyses, data should be of the normal distribution. Normal distribution can be obtained by transformation of the raw data either by use of averages or in this case by $y = \log_{10} (X + 1)$. Averages, and in particular non-normal data, when used in statistical methods of analyses, tend to gloss over or obscure points of difference. This is particularly true in the analysis of variance when the results are based on the distribution of F.

The timing of sampling for the early larval populations was fairly consistent with the standards set forth (table 2). The late larval and pupal sampling was not as well timed, but generally was performed within a few days of the proper developmental period. Rearing collected individuals probably offset this effect. Timing of collections was designed to obtain an accurate and complete parasite picture. The trend of the budworm parasites and seasonal defoliation for the past 3 years is portrayed graphically in figure 1.

Table 2.--Budworm population by instars at time of sampling (1958-1960)

Plot	Year	Sample	Instars by Percent 2	3	4	5	6	P	A	Total No. individuals
EN-1	1958	Early	17	61	22	< 1	0	0	0	123
	1959		23	50	21	6	0	0	0	166
	1960		13	65	22	0	0	0	0	55
EN-2	1958	Early	23	73	4	0	0	0	0	47
	1959		57	39	4	0	0	0	0	44
	1960		18	82	0	0	0	0	0	11
EX-4	1958	Early	8	31	35	17	9	0	0	214
	1959		33	50	10	5	2	0	0	40
	1960		no sample taken							
EN-1	1958	Late	0	0	6	11	22	39	22	18
	1959		0	0	4	18	40	38	0	45
	1960		0	0	0	0	0	67	33	3
EN-2	1958	Late	0	0	0	26	42	26	5	19
	1959		0	0	0	33	67	0	0	9
	1960		0	0	25	0	25	50	0	4
EX-4	1958	Late	0	0	19	15	30	18	18	25
	1959		0	0	0	0	0	100	0	1
	1960		0	0	0	0	0	100	0	1

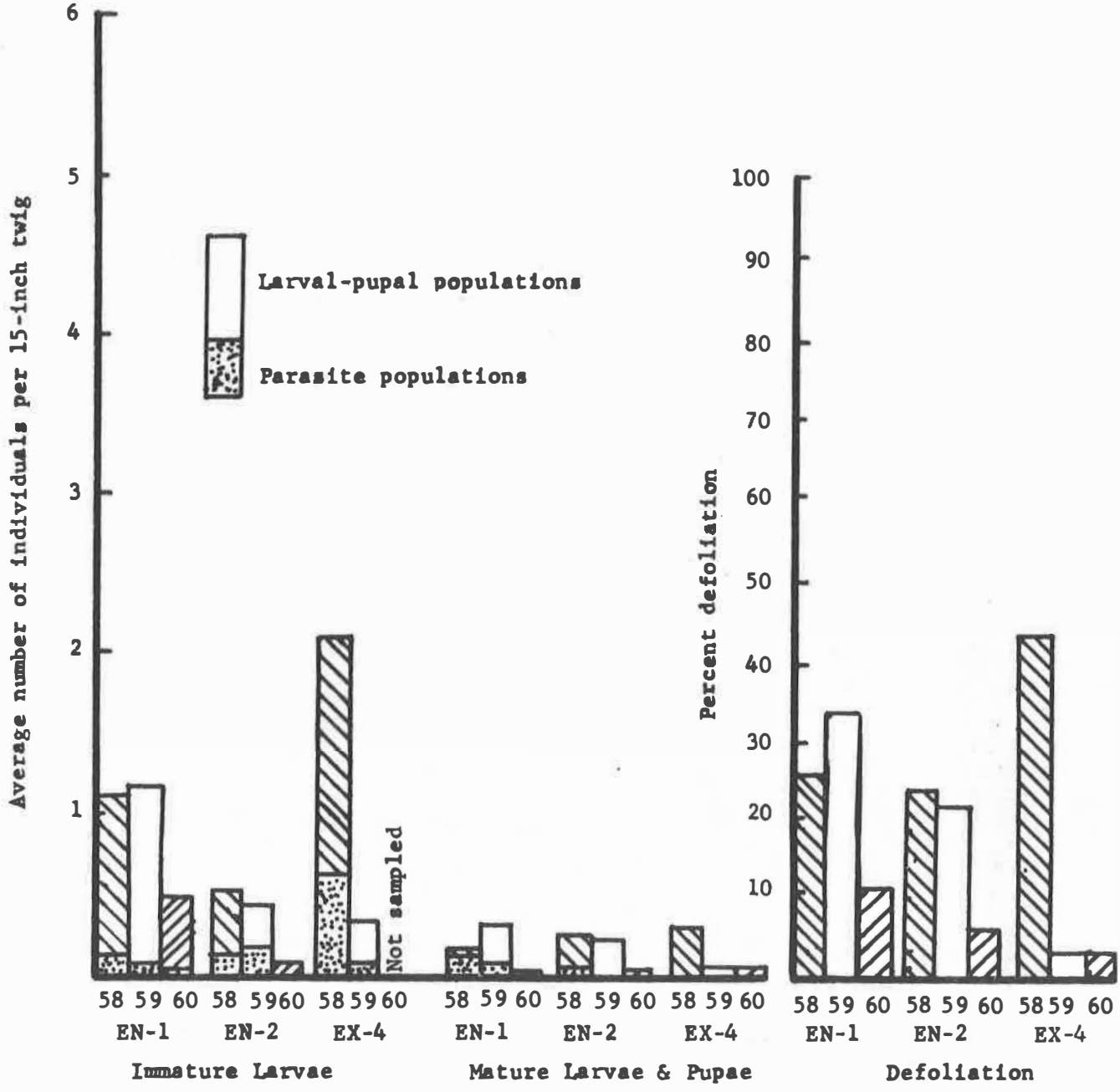

Figure 1.--Larval and parasite populations and defoliation (1958-1960).

In order to follow a population from year to year, or even from stage to stage, it is necessary to test the relation between years and stages. The plots had been chosen on the basis of age and character of the infestations rather than abundance of individuals. A test of independence was utilized to compare early and late populations within years and between years within each tree age class, by plots. However, the results showed no significant difference in the early larval populations per twig between years, late larval populations per twig between years, nor amount of reduction between early and late populations between years. In other words, the populations approximated each other in the same plot each year--no change in population during the study period. Close scrutiny of the averages revealed a slight downward trend, but for all practical statistical measures, the distinction between years may be discarded and the larval population data pooled as an unbiased estimate for other analyses.

Parasites were inventoried and the percent of parasitism recorded throughout the study years. The parasite population remained at a low level, decreasing in proportion to the host trend on the study plots (table 3).

Table 3.--Parasites and percent parasitism within the spruce budworm larval and pupal populations (1958-1960)

Sample	Plot	Year	Percent by Parasites: Apanteles	Glypta	Horogenes	Total percent	Total number
Early	EN-1	1958	4	2	1	7	8
		1959	2	1.5	0	3.5	4
		1960	2	2	0	4	2
	EN-2	1958	11	7	0	18	8
		1959	8	22	0	30	11
		1960	0	0	0	0	0
	EX-4	1958	21	10	<1	32	67
		1959	3	6	0	9	3
		1960	no sample taken				

Sample	Plot	Year	Bracon poliventris	Ichneumonini sp.	Madremyia saundersii	Actia affinis	Other	Total percent	Number
Late	EN-1	1958	0	6	0	0	25	31	5
		1959	3	0	3	0	0	6	2
		1960	0	0	0	0	0	0	0
	EN-2	1958	0	0	0	0	11	11	2
		1959	0	0	0	0	0	0	0
		1960	0	0	0	0	0	0	0
	EX-4	1958	0	0	0	5	0	5	1
		1959	0	0	0	0	0	0	0
		1960	0	0	0	0	0	0	0

Methods were developed during the study years for sampling any one of the stages--immature larvae, mature larvae and pupae, emerged pupal cases, and total seasonal defoliation. Subsequently, relations were tested between populations within different tree age classes.

In addition to merely sampling immature larval populations, the data gathered were tested and a significant correlation found to predict the total seasonal defoliation. The three years of data were used to compute the following regression equation:

$$\text{Log } (y + 1) = 0.18606 + 1.60110 \left[\overline{\text{Log } (x + 1)}\right]$$

Where: y = expected percent of defoliation
x = number of immature larvae

This equation is graphically shown in figure 2 and its field operative form in table 4. The data from biological evaluation are plotted on figure 2 to show the accuracy of this prediction table in use.

In order to evaluate an extensive budworm infestation, information of the population can best be obtained by sampling trees of all age classes. This can be laborious and time consuming. Consequently, it seemed desirable to test the correlation and relation of larval populations within trees between age classes. In addition, if the understory could be sampled, and that population projected to the overstory, pole pruners could be discarded and replaced by hand pruners. Thus, ease of movement through the woods would be increased with a savings in time and labor.

The reproduction age class was excluded due to the high variation of the budworm larval population within the sample unit. Sampling of immature and mature trees usually requires pole pruners while some pole and most sapling sized trees can be hand pruned for samples. Thus, attempts to relate larval populations between pole and mature as well as sapling and mature, were made during sampling of immature larvae and mature larvae and pupae.

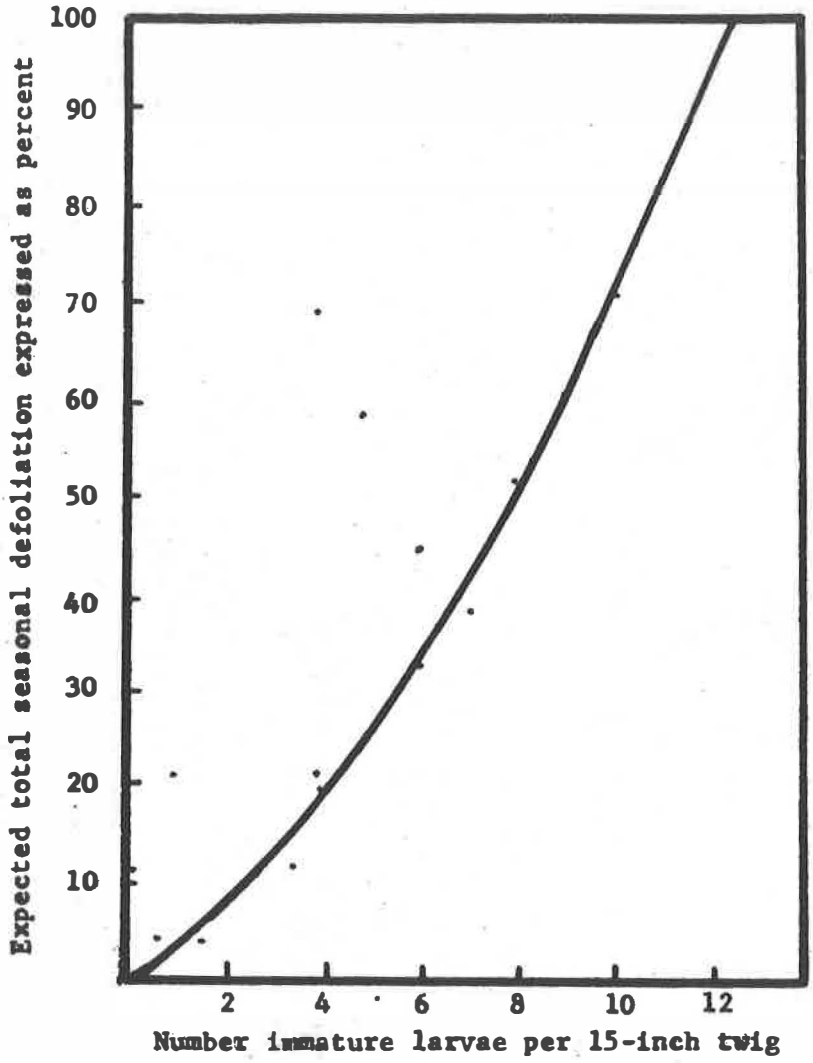

Figure 2.--Regression of total seasonal defoliation on immature budworm larval populations.

Table 4.--Table for field use of prediction of total seasonal defoliation based on immature larval populations per 15-inch twig.

Average number larvae per twig	Expected percent of defoliation
1-5	< 25
6-8	25-50
9-10	50-75
11-12	75-90
>13	>90

Significant regressions were computed and followed these equations:

Larval population stage	Tree age class	
Immature	sapling vs. mature	$\text{Log } (y+1) = 0.428724 + 1.04725 \overline{/\text{Log } (x+1)/}$
	pole vs. mature	$\text{Log } (y+1) = 0.11390 + 0.03033 /\text{Log } (x+1)/$
Mature	sapling vs. mature	$\text{Log } (y+1) = 0.28468 + 0.047320 \overline{/\text{Log } (x+1)/}$
	pole vs. mature	$\text{Log } (y+1) = 0.22148 + 0.63660 /\text{Log } (x+1)/$

These equations were computed from the pooled data obtained during several years in study plots and were tested against data collected from the biological and evaluation and study plots. However, when biological evaluation data were plotted to determine fit, the curve failed completely.

Numbers of egg masses per sample unit have been used successfully in other areas to follow and predict budworm infestation trends. Spruce budworm egg masses have been extremely difficult to find and sample with reliability in southern Idaho. Consequently, the next preceding stage, emerged pupal cases, was investigated and tested for correlation with the immature larvae of the following spring. A significant regression was computed in 1959 using 1958 emerged pupal cases against 1959 immature larvae. Field tests were conducted in 1960 and the prediction equation proved reliable. Pooled data, 1958 and 1959 emerged pupal cases vs. 1959 and 1960 immature larvae, gave a significant regression following the equation:

$$\text{Log } (y+1) = 0.27148 + 1.5689 \overline{/\text{Log } (x+1)/}$$

Where: y = expected number of immature larvae
x = number of emerged pupal cases

This is not greatly different from the previously calculated equation. Figure 3 shows this regression in graph form and compares the actual biological evaluation data for the Salmon National Forest infestations and Fleck Summit on the Sawtooth National Forest. The regression is shown in field operative form in table 5.

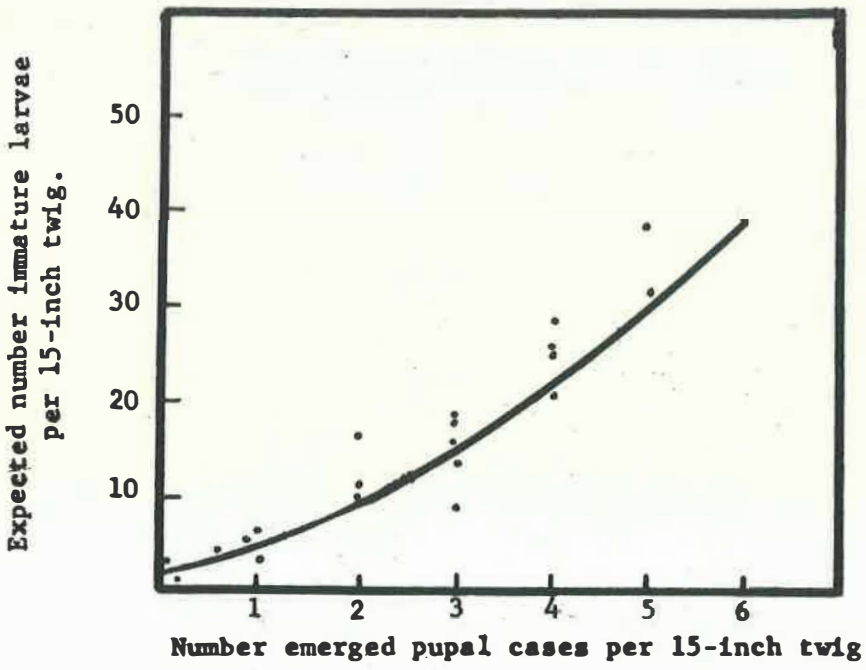

Figure 3.--Regression of immature budworm larvae on emerged budworm pupal cases on Douglas-fir (2, 3, 4 instars).

Table 5.--Field operative form for prediction of immature (2-3-4 instar) budworm larvae based on emerged pupal cases per 15-inch Douglas-fir twig.

Average number emerged pupal cases per twig	Expected number immature larvae per twig
< 0.5	< 2.5
1	2.5 - 7.0
2	7.0 - 12.5
3	12.5 - 19.0
4	19.0 - 26.0
5	> 26.0

These data were taken from populations that had remained for 3 years at a level of abundance that caused approximately 50 percent defoliation. Consequently, a sudden change in populations (post pupal stage) could disrupt this prediction. In fact, just such a change did occur in Montana during 1960.[1/] Biological evaluation data taken in 1959 and 1960 concerning the egg mass stage were compared (table 6). As shown, all factors were comparable except egg masses per thousand square inches of foliage.

Table 6.--Comparison of 1959 and 1960 egg mass data (Montana)

Factor compared	1959	1960
Percent defoliation	38	34
Moths/15-inch twig	1.11	0.95
Eggs/egg mass	41.97	40.84
Egg mass/1000 sq. in. of foliage	10.36	3.63

Thus, even as late as egg deposition, fluctuations can occur that would upset the best prediction. A comparable moth population level in 1959 and 1960 showed a comparable number of eggs per mass, yet a 65 percent reduction in numbers of egg masses occurred in 1960 over those recorded in 1959. The specific cause of reduction is unexplainable at this time.

Early in the study, a method was developed to force emergence of overwintering larvae by fluctuating time and temperatures.

Billets containing hibernacula were stored at (1) 40° F. for 1 week, (2) -25° F. for 8 hours, and (3) then removed to 68°-70° F. until emergence was completed. Under these conditions larvae began emergence 22 days after removal from the -25° F. temperature. Cumulative temperature at the end of these 22 days averaged 684 degrees or a daily average gain of 33 degrees.

BIOLOGICAL CONTROL

Objective

To test the effectiveness of a possible budworm virus

Methods

During the course of study, what appeared to be a virus disease was discovered affecting budworm. Cage tests were made to determine its virulence as a control agent. Three concentrations were used against third and fourth instar larvae. Application of the virus solutions were made with a calibrated atomizer at a rate comparable to 1 gallon per acre. Mortality results were recorded.

1/ Terrell, T. T. Personal communication.

Results

Several suspected diseased budworm larvae were collected after the completion of the 1957 budworm control project. These larvae were removed from cold storage in 1959, made into a solution, and applied in a cage test at the rate of 3.5×10^7 polyhedra per milliliter per acre with a calibrated atomizer. The test indicated that 61 percent of the larvae died from virus infection. Forty-one ml. of concentrate containing 1.35×10^8 polyhedra per ml. were collected from this experiment.

In 1960, the same concentration and spray rate was used against larvae collected in Montana and Idaho. No mortality resulted due to virus, in either case.

DAMAGE

Objective

To determine the role of residual old-growth needle complement following defoliation, and relate its importance to tree survival.

Methods

The needle complement phase of the study was designed to determine the relation of old-growth needles to tree survival. The theory being that the capability of a tree to survive defoliation, or conversely the effect of defoliation on a tree, could be measured through its needle complement.

During 1958, the first year of this study, the needle complement on 15-inch twigs was compared with that of twigs including 6 years of growth. These samples were taken from different age-class trees that had suffered different degrees of defoliation within the study plots. The analysis showed that a Douglas-fir twig consisting of 6 years of growth was a reliable sample.

Analysis of the data collected in 1959, using green, nondefoliated, 6-year twigs, showed that possibly up to 50 percent defoliation had no appreciable effect on the needle complement when related to natural needle droppage. The main difference in needle complement, based on needles per twig inch per year, existed between trees within pole and immature age classes.

In 1960, specific needle complement plots were established. These plots consisted of 10 pole, and 10 sapling-age Douglas-fir trees that had received varying degrees of defoliation. The defoliation classes, less than 25 percent and 25 to 50 percent, were combined and designated as less than 50 percent defoliation. Four plots were established and trees within the plots were carefully chosen to include examples of selected degrees of defoliation.

Results

The needle complement phase utilized natural rather than mechanical defoliation. For this type of study the possible combinations of defoliation for 6 years is not only overwhelming, but any method used to simulate budworm conditions would be too laborious. The only disadvantage in natural defoliation is that all intensities of defoliation are not readily available.

An analysis of variance was used to test differences and sort out their causes in both twig growth and needle complement. Five main sources of variation were used in each test:

1. Amount of defoliation (4 classes based on number of new growth tips destroyed).

2. Tree age - pole and sapling per defoliation class.

3. Plot site - good and poor, per tree age.

4. Trees - 5 per site.

5. Growth - 6 years per twig (two twigs per tree).

It was interesting to note, only the site influenced the amount of linear growth of a 6-year twig. As suspected, the better site produced the greater linear growth. However, when the influence of tree age was combined with either amount of defoliation or individual tree, significant interactions resulted. In both cases the significant differences were due to inconsistency of the sums for each defoliation class x tree age interaction and for each tree age x individual tree interaction (tables 7 and 8).

Table 7.--Sums for the defoliation by tree age interaction

	Defoliation Class			
Tree age	< 50%	50-75%	75-90%	> 90%
Pole	477.20	447.56	696.45	698.19
Sapling	558.55	359.69	661.34	554.61

Table 8.--Sums for the tree age by individual tree interaction

	Individual tree				
Tree age	1	2	3	4	5
Pole	442.78	582.94	306.39	572.92	414.37
Sapling	628.13	695.23	428.79	406.74	424.10

On the other hand, site played no part in differentiating the residual needle complement (average number of residual needles per linear inch of twig growth per year).

In 1959, the influence of tree age on the needle complement of green, nondefoliated trees was shown to exist. This difference in needle complement could be separated into two main groups: pole-mature-reproduction group and sapling-immature group. There was no difference in needle complements within either of the two groups. This difference was again shown in 1960, but in this case only pole and sapling age classes were used. Remarkably, the addition of the influence of defoliation did not change the situation.

As expected, total complement varied between defoliation classes. However, the total needle complement did not vary proportionally with the amount of defoliation (table 9). This was due to the confounding of different degrees of defoliation each year for six years.

Table 9.--Total needle complement by defoliation class

Total needle complement	Defoliation Class			
	< 50%	50-75%	75-90%	>90%
6 years	2041.70	1390.46	2066.85	634.24
0-year	699.59	454.40	216.10	51.91

If the 6 years totals are separated by their component years of needle complement the inconsistency becomes evident (table 9). Only in the current year's complement (0-year) does the expected pattern appear. Consequently, one year sampling of either new growth tips or needle complement could not properly evaluate the effect of defoliation on tree survival. Such an evaluation would necessarily be extremely limited. The evaluation could possibly take on meaning through cumulation of current data taken annually for six years.

The assumption was made that a survival scale might be devised, based on a cumulative sum of weighted values for each age needle complement, that would show a range from tree death to normal tree growth. This value would be designated as the Needle Complement Value (NCV) for a particular tree or group of trees. The calculation of the NCV is shown in table 10. As an example, based on the exploratory results of a few workers in the field of tree physiology, arbitrary values were assigned to the individual needle according to its photosynthetic importance by age.

The needle complement values were calculated for green, nondefoliated pole and sapling age trees. For comparison purposes, values were also calculated on the speculative basis that these trees had received 25, 50, 75, and 90 percent defoliation each year for the past six consecutive years (table 11).

Table 10.--Needle ranks and method for determining the needle complement value (NCV) of Douglas-fir

Needle age	Rank	Average needles per inch	Needle complement value
New or current	0.6	n_o	$0.6n_o$
1 year old	1.0	n_1	$1.0n_1$
2 " "	0.6	n_2	$0.6n_2$
3 " "	0.4	n_3	$0.4n_3$
4 " "	0.3	n_4	$0.3n_4$
5 " "	0.2	n_5	$0.2n_5$
			Sum = NCV for sample

Table 11.--Needle complements and needle complement values of green, nondefoliated trees and different degrees of defoliation for six consecutive years

Age of needle	Photosynthetic value	Needle complement by defoliation for 6 years — Pole					Sapling				
		0	25	50	75	90	0	25	50	75	90
0	0.6	50.38	37.79	25.19	12.60	5.04	65.61	49.21	32.86	16.40	6.56
1	1.0	31.98	23.99	15.99	8.00	3.20	39.95	29.96	19.98	9.99	4.00
2	0.6	32.42	24.32	16.21	8.11	3.24	35.32	26.49	17.66	8.83	3.53
3	0.4	24.66	18.50	12.33	6.17	2.47	41.86	31.40	20.93	10.47	4.19
4	0.3	18.96	14.22	9.48	4.74	1.90	25.62	19.22	12.81	6.41	2.56
5	0.2	15.25	11.44	7.63	3.81	1.53	21.58	16.19	10.79	5.40	2.16
N C Value		100.26	75.21	50.13	25.07	10.03	129.26	96.94	64.63	32.32	12.93

- 17 -

The values shown are probably more realistic than they first appear, particularly the needle complement values for 90 percent defoliation for six years. Table 12 shows actual needle complement values from the sample trees, by plots. The defoliation class they would fall in can be compared with values in table 11. Note the small number of needles per inch of twig growth per year and yet these trees are surviving. The most interesting note is the greatest and probably most significant reduction in needle complement values occurred between the greater than 90 percent defoliation class and the other three classes. Site 1 was the poor site, site 2 the good site.

Table 12.--Needle complements and needle complement value of study trees by site and percent defoliation occurring in 1960

		Needle complement by current defoliation class							
		Site 1				Site 2			
Tree age	Needle age	<50	50-75	75-90	>90	<50	50-75	75-90	>90
Pole	0	35.90	25.50	12.57	1.89	31.16	17.66	8.73	3.00
	1	23.60	18.47	13.81	0.66	18.47	6.41	37.10	0.67
	2	8.39	11.62	15.87	3.76	9.07	3.08	27.62	4.86
	3	17.17	16.76	12.76	4.75	17.31	11.93	15.76	11.62
	4	14.80	9.54	18.91	8.23	15.78	8.65	19.86	12.91
	5	18.35	2.88	17.48	5.30	11.75	9.75	17.36	1.22
N C Value		65.16	50.88	50.16	9.48	56.62	28.17	74.64	14.15
Sapling	0	32.24	25.91	7.67	2.63	40.62	21.81	14.04	2.87
	1	18.28	16.69	11.58	0.11	13.35	12.43	21.19	0.65
	2	5.77	3.93	17.93	2.85	8.60	4.98	17.70	5.93
	3	16.63	11.56	9.90	8.74	12.54	7.83	13.12	14.92
	4	14.34	10.79	19.55	7.91	4.27	7.80	16.39	15.21
	5	19.09	3.45	20.13	2.00	1.64	8.66	21.33	4.18
N C Value		55.86	43.15	40.79	9.67	49.51	35.71	54.66	17.34

Site, as previously stated, did not exert detectable influence on needle complement and apparently this is true when carried on through the needle complement value.

In addition to loss of needles due to defoliation, annual natural droppage of needles enters into the picture. The rate of needle retention was compared between defoliated and nondefoliated trees in 1959 (figure 4). This was done in hopes of revealing a little more information on the true effect of defoliation--i.e., if a nondefoliated tree is growing with apparently good vigor at a particular needle complement value, including natural needle drop, just how much influence does defoliation have on the growth of the tree beyond this level of needle drop?

Since the analysis showed only one case of significant difference in needle complement by tree age class, data was grouped by defoliation class (figure 4).

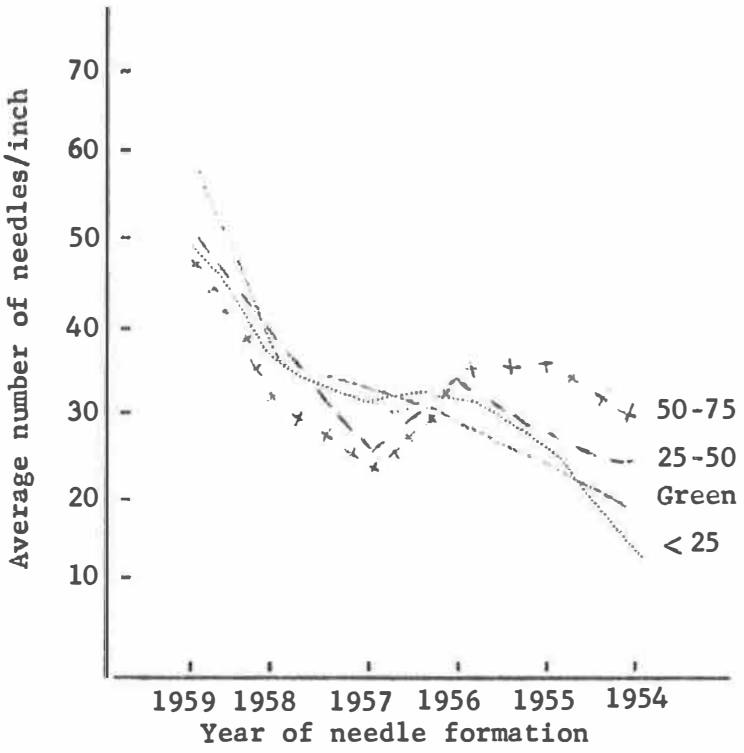

Figure 4. Rate of needle retention between non-defoliated and defoliated Douglas-fir trees.

Curvilinear regressions were then computed for rate of needle retention for green trees, defoliated trees, and a common regression between the two. In each case the regression on X_1 (age of needle) was highly significant, thus indicating a definite difference in needle complement by year (figure 5).

These regressions followed the formulae:

$$\text{Common} \quad Y = 19.66042 - 5.28994X_1 + 1.87943X_2$$
$$\text{Green} \quad Y = 31.00841 + 0.01602X_1 + 1.28944X_2$$
$$\text{Defoliated} \quad Y = 13.92676 - 2.55177X_1 + 1.14881X_2$$

Where: Y = average number of needles/inch
X_1 = year of needle formation
X_2 = year of needle formation squared

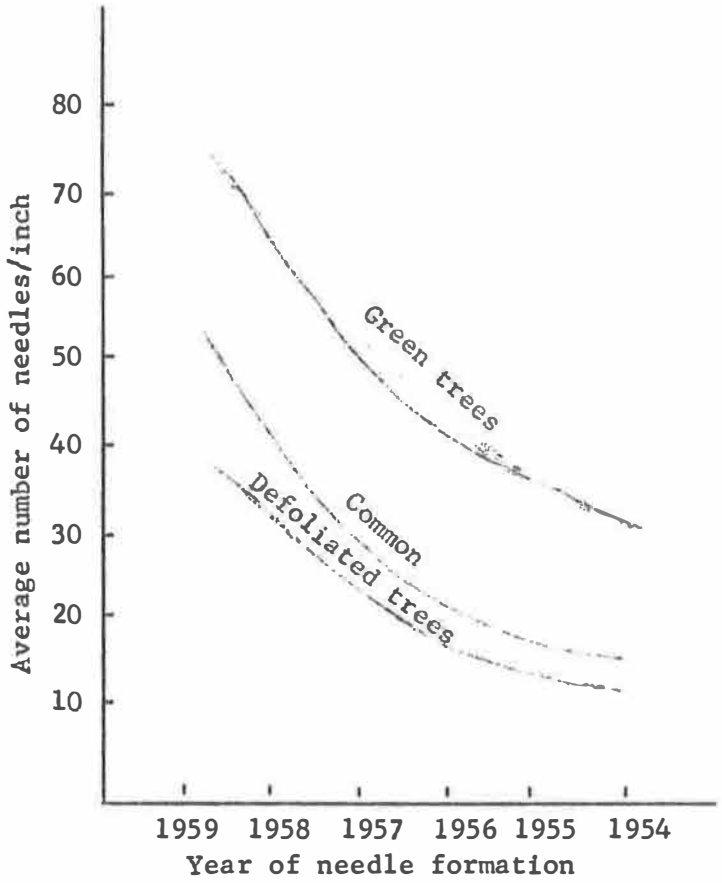

Figure 5.--Regressions of rate of needle retention on age of needle for Douglas-fir.

An analysis of variance was then made to test if there was any real difference between the green and defoliated regressions. The calculated F was close enough to the required F value so that it may be stated that a significant difference does exist between these two populations (table 13). However, this may also suggest that up to the 50 percent defoliation class there could be no effect to the needle complement.

Table 13.--Analysis of variance for differences in the regressions of needle complement between green and defoliated trees

Source	DF	M. sq.	F	Reg. F
Residual of common regression	57	57.09	1	
Differences between green and defoliated regressions	3	175.65	2.73	2.78*
Residuals of green and defoliated regressions	54	64.40		

DISCUSSION

Possibly the best approach to discussing the results of this study is to describe their use in making a biological evaluation of a spruce budworm infestation.

Five sample plots per infestation should suffice for adequate coverage. A sample plot should consist of 20 trees. One plot is to be taken from the center of the infestation (not necessarily the geographic center) and four plots scattered immediately within the periphery of the infestation. The same plots should be utilized for each sample during any one year. The first sample should be taken when approximately 50 percent of the emerging larval population has reached the third instar; the second sample when 50 percent of the larval population has pupated; and the third sample when adult moth emergence and defoliation is complete. The timing of the first two samples is highly important to obtain parasite and disease information.

The first sample (immature larvae) should consist of 4, 15-inch twigs from each of 20 trees. This sample size should suffice for populations averaging one or more larvae per twig and the results should approximate the accuracy of 20 percent SME at a 90 percent confidence level. The other estimates, mature larvae and pupae, emerged pupal cases and defoliation, may be obtained with the use of the sequential plans. This plan was calculated at the 90 percent confidence level for each stage to be sampled. Computation of the needle complement value is derived from 2 twigs, each containing six year's growth, from each of 10 trees.

All tree age classes should be represented in each sample.

It is realized that in dealing with many and varied infestations and a small working force the ideal multi-visit sampling cannot always be accomplished. Thus, relations were developed between samples to aid the evaluator and add supplemental data.

Results from sampling the immature larval population shows the original seasonal population level and can be used to relate or predict the total seasonal defoliation in case further sampling is not practicable. Normally, it is easier to dissect the larvae from this collection rather than rear them to inventory species and determine the number of parasites present.

The second sample, mature larvae and pupae, reflects the population reduction, index of the population level, and by rearing these individuals the parasite influence is recorded. This estimate is found by use of the sequential plan[1/] and is also the one used in evaluating spruce budworm control projects.

1/ Cole, Walter E. Sequential Sampling in spruce budworm control projects. Forest Science, Vol. 6, No. 1, March, 1960.

Either or both the immature larval and mature larval-pupal sample can be used in a test of independence to determine the trend of infestation within and between years.

In addition, these are the stages most likely to reflect a disease should one become active in a budworm infestation.

The third sample, emerged pupal cases and total seasonal defoliation, is probably the most important sample. The sequential plan can be utilized for both populations.[2/] Emerged pupal cases can be used to predict the following spring larval population. Caution should be used in following a prediction based on pupal cases at this time. Further work is needed in highly fluctuating infestations before the time consuming egg mass surveys can be eliminated in other than static infestations. However, egg masses can be recorded at the same time, thereby adding information of one more stage.

A biological evaluation conducted along these lines should show:

(1) Population reduction during the season.
(2) Causal parasitic agents and possible diseases - larval and parasite.
(3) Population trend between years.
(4) Total seasonal defoliation.
(5) Prediction of the immature larval population for next year.

2/ Terrell, T. T. Personal communication

APPENDIX

During the year, prior to the establishment of the 1/10-acre plot, groups of five trees were selected and sampled. This exploratory work set the sampling pattern for the more intensive work that followed. Sample size, sampling location within the tree crown, and frequency of sampling were determined. This sampling showed the following:

(1) No significant population variation between 2nd through 5th instars, but a highly significant difference between these stages and the last two instars (6th and pupae).

(2) No significant difference in larval populations existed between mid- and top-crown, but a high variation existed between population in the low crown as opposed to mid- and top-crown.

(3) Buds, or new growth tips, were evenly distributed per 15-inch twig throughout the crown of Douglas-fir, but the bud distribution within alpine fir paralleled the larval distribution (#2).

(4) There was an average of 24.90 buds (new growth tips) per 15-inch twig on Douglas-fir and 96.90 on alpine fir.

(5) Total buds, or new growth tips, partially or completely eaten, per larvae, were 8.98 on Douglas-fir; 8.64 on alpine fir. Yet, during this year (1957) percent defoliation was 35.06 and 85.06 respectively.